

MORPHOLOGICAL AND CYTOLOGICAL EVIDENCE FOR HOMOPLOID HYBRIDIZATION IN *IOCHROMA* (SOLANACEAE)

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ABSTRACT

Previous phylogenetic and biogeographic studies of *Iochroma* (Solanaceae) suggested that *I. ayabacense* S. Leiva is a hybrid between *I. cyaneum* (Lindl.) M. L. Green and *I. lehmannii* Bitter. Chromosome counts for these three taxa demonstrate that all have a haploid chromosome number of $n = 12$, and thus that the formation of *I. ayabacense* did not involve changes in ploidy level. A comparison of vegetative and floral morphology revealed that *I. ayabacense* demonstrates a striking intermediacy between *I. cyaneum* and *I. lehmannii*. The results support the conclusion that *I. ayabacense* is a homoploid hybrid between *I. cyaneum* and *I. lehmannii*.

RESUMEN

Estudios filogenéticos y biogeográficos de *Iochroma* han sugerido que *I. ayabacense* S. Leiva es un híbrido entre *I. cyaneum* (Lindl.) M. L. Green y *I. lehmannii* Bitter. Recuentos de cromosomas definen un número haploide de $n = 12$ para estas especies y así indican que la formación de *I. ayabacense* no involucró un cambio en el nivel de ploidía. Una comparación de la morfología vegetativa y floral reveló que *I. ayabacense* tiene un forma intermedia entre *I. cyaneum* and *I. lehmannii*. Estos resultados respaldan la conclusión que *I. ayabacense* es un híbrido homoploide entre *I. cyaneum* y *I. lehmannii*.

Key Words: Amotape-Huancabamba, chromosome number, homoploid hybridization, *Iochroma*, Solanaceae.

Interspecific hybridization has long been recognized as an important generator of diversity in flowering plants (Grant 1981; Rieseberg 1997). Hybrid speciation can occur via two modes: allopolyploidy, in which the hybrid exhibits twice the chromosome number of its parental species, and homoploidy, in which the hybrid has the same ploidy as both parents (Arnold 1997). Allopolyploidy is considered to be the more common mode of hybrid speciation because the change in chromosome number creates an instant reproductive barrier between the hybrid and its parents, facilitating persistence of the hybrid species (Ramsey and Schemske 1998; Chapman and Burke 2007). However, theoretical work suggests that homoploid hybridization can also lead to stable hybrid lineages (Buerkle et al. 2000), either through recombinational speciation (Stebbins 1959; Grant 1981) or through ecological selection favoring novel hybrid phenotypes (Rieseberg et al. 1999). While the number of clear examples of homoploid hybrid speciation remains small (Rieseberg 1997), combining molecular phylogenetic studies with traditional lines of evidence holds promise for detecting additional instances (e.g., Ferguson and Sang 2001; Howarth and Baum 2005). Here we apply cytological and morphological evidence to examine the

case of *Iochroma ayabacense*, a species suspected of hybrid ancestry based on recent molecular phylogenetic studies (Smith and Baum 2006).

Iochroma is a genus of approximately 25 species distributed throughout the northern Andes, from Colombia to Peru. Based on conflicting placement across gene trees, Smith and Baum (2006) identified three suspected hybrid taxa in *Iochroma*, of which *I. ayabacense* provided perhaps the most compelling case. In phylogenetic analyses of three nuclear regions (ITS, *LFY* intron II and *waxy*), *I. ayabacense* was found to contain “divergent” alleles, which fell either in the *I. cyaneum* group or in the *I. lehmannii*-*I. squamosum* group (Smith and Baum 2006). We consider *I. squamosum* S. Leiva and Quipuscoa a synonym of *I. lehmannii* and will treat it as such in this paper. This phylogenetic information together with the geographic distribution of *I. ayabacense* strongly suggested that it is the product of hybridization between *I. lehmannii* and *I. cyaneum*. In this paper, we examine the morphological features of *I. ayabacense* relative to its putative parents, and report on the results of chromosome counts undertaken to determine whether the formation of *I. ayabacense* involved a change in ploidy level.

METHODS

I. ayabacense occurs exclusively in the environs of Ayabaca, Peru, near populations of both *I.*

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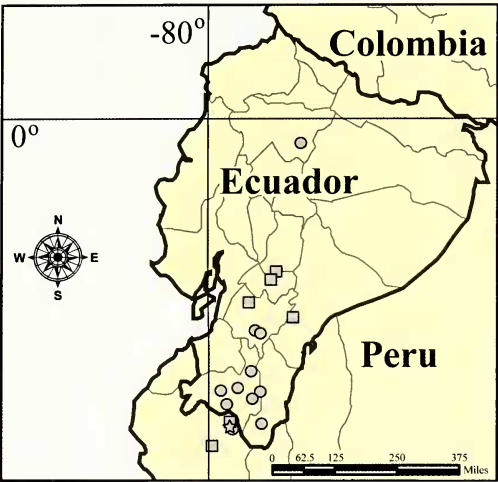


FIG. 1. Geographic distribution of study taxa. Circles are sites where *I. cyaneum* has been collected and squares where *I. lehmannii* has been collected. *I. ayabacense* is only found in Ayabaca, Peru, indicated by the star.

cyaneum and *I. lehmannii* (Fig. 1). Buds for chromosome counts were collected from individuals of *I. ayabacense* and *I. lehmannii* near Ayabaca, and buds from *I. cyaneum* were collected from a greenhouse-grown accession. Vouchers for these sources are given in Appendix 1. The buds, ranging from 4–20 mm in length, were fixed in Carnoy’s solution (3 parts chloroform: 2 parts ethanol: 1 part acetic acid) for 12–24 hr and then transferred to 70% ethanol for storage. Slides of meiocytes stained with acetocarmine were prepared using the “squash” technique outlined by Beeks (1955) and modified by Kowal (1975) and viewed with a Zeiss standard WL microscope with a 100× oil immersion objective. Twelve cells with chromosomes clearly visible in a single plane were counted for each species. Images of meiotic figures from each species were captured with an Axiocam Hrm mounted on a Zeiss Axioplan 2 microscope using the 100× oil immersion objective.

Information on floral and vegetative morphology of *I. ayabacense* and its putative parents was compiled from taxonomic descriptions (Bitter 1918; Shaw 1998; Leiva et al. 2003; Leiva and Lezama 2005) and observations of all available specimens (see Appendix 1). Fourteen morphological traits (see Appendix 2) were scored for the available specimens of the three taxa, and these data were analyzed using principal components analysis in JMP 7.0 (SAS Institute Inc., Cary, NC).

We also measured pollen grain size, which is known to be positively correlated with ploidy level (Gould 1957; Kowal 1975). Measurements of mature pollen grains were taken from individuals used for chromosome counts. Pollen was

stained with gelatin fuchsin, and twenty pollen grains from each individual were measured to the nearest 0.1 μm using a Zeiss AxioSkop 2 microscope with a 40× objective.

RESULTS

Anthers at roughly two-thirds their mature length (2.2 mm for *I. cyaneum*, 2.1 mm for *I. ayabacense* and 3.5 mm for *I. lehmannii*) provided the best material for chromosome counts. Within a single flower containing anthers at this size, we were able to observe all the stages of meiosis I and II. Some cells could be counted at prophase I and II, but the most countable stage for all species was telophase II. Chromosome counts for all species including *I. ayabacense* were $n = 12$ (Fig. 2), consistent with previous counts in *Iochroma* [*I. cyaneum*, $n = 12$ (Ratera 1961; Mahadavian 1967; Mehra and Bawa 1969) and *I. fuchsoides*, $n = 12$ (Ratera 1961)]. Pollen size for *I. ayabacense* fell within the range of the parents (Table 1), consistent with the fact that all three share the same ploidy level (Gould 1957; Kowal 1975).

The vegetative and floral features of *I. ayabacense* appeared to be largely intermediate between its putative parents (Table 1). The plant habit, amount of pubescence, numbers of flowers per inflorescence, and the form of the flowers (Fig. 2) fell exactly between *I. lehmannii* and *I. cyaneum*. Size measurements (e.g., plant height) for *I. ayabacense* were within the ranges observed in the putative parents although, for a few traits, such as leaf length, *I. ayabacense* exhibited a wider range of variation than the parents. Also, in some traits, *I. ayabacense* favored one of the putative parents. For instance, it largely lacks the stem scales characteristic of *I. lehmannii*, but its fruits are nearly included in the accrescent fruiting calyx, resembling *I. lehmannii* rather than *I. cyaneum*.

Principal components analysis (PCA) provided additional evidence for the morphological intermediacy of *I. ayabacense*. Variation across the 14 floral and vegetative traits (Appendix 2) was largely captured by the first few components, with components 1 and 2 accounting for 73% (Fig. 3). The components clearly separate the tree taxa into discrete clusters in morphospace with *I. ayabacense* falling between its putative parents (Fig. 3).

DISCUSSION

This cytological and morphological study, together with existing phylogenetic and biogeographic information, shows that *I. ayabacense* is a homoploid hybrid between *I. cyaneum* and *I. lehmannii*. While the phylogenetic data (Smith and Baum 2006) provided clear evidence that *I. ayabacense* carries alleles derived from both the *I. cyaneum* lineage and the *I. lehmannii* lineage, they

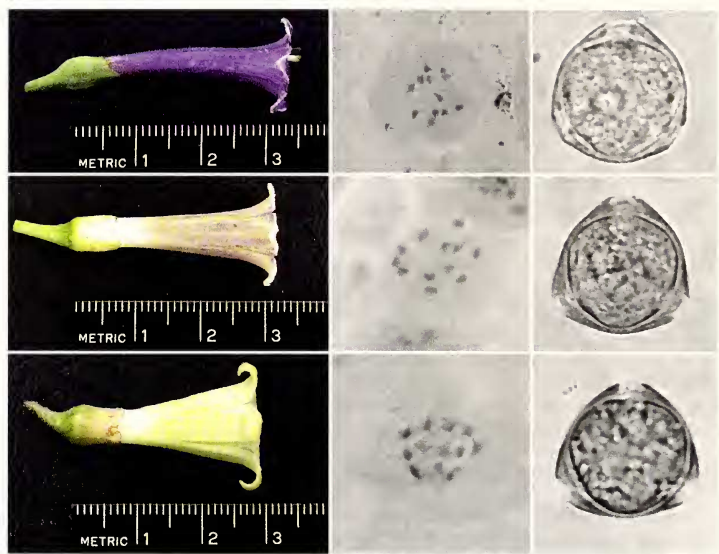


FIG. 2. Flowers, meiotic figures and pollen grains from *I. cyaneum*, *I. ayabacense* and *I. lehmannii* (top to bottom). The haploid number of $n = 12$ chromosomes is visible in all three taxa.

did not indicate whether *I. ayabacense* was a homoploid or polyploid hybrid. The chromosome counts and the pollen measurements presented here indicate that *I. ayabacense* has the same ploidy level as its parents ($n = 12$). Also, the morphological data demonstrate that *I. ayabacense* is intermediate between its two parents in a wide array of floral and vegetative traits. Although the morphological distinctiveness and cohesiveness of *I. ayabacense* was deemed

sufficient to merit specific recognition (Leiva and Lezama 2005), it remains to be seen whether the species can maintain reproductive isolation from its parents. At present, populations of *I. ayabacense* are largely separate from populations of *I. cyaneum* and *I. lehmannii*, perhaps due to differences in microhabitat preferences (Smith and Baum 2006). However, extensive searching during a recent collecting trip revealed one apparent backcross individual (*I. ayabacense* ×

TABLE 1. MORPHOLOGICAL DIFFERENCES AMONG *I. CYANEUM*, *I. AYABACENSE* AND *I. LEHMANNII*. ¹ This range is larger than that given in the species description, which appears to have been based on immature fruits.

<i>I. cyaneum</i>	<i>I. ayabacense</i>	<i>I. lehmannii</i>
Shrub, 1.5–3 m	Shrub to small tree, 3–4 m	Small tree, 4–10 m
Young stems green, densely covered in branched hairs	Young stems green or purplish toward the tips, covered in branched hairs, with occasional small triangular scales	Young stems purplish and covered in triangular scales
Ovate to elliptic, 10–23 cm by 5–10 cm	Elliptic to lanceolate, 8–22.5 cm by 5–10.5 cm	Elliptic to lanceolate, 4–18.5 cm by 2.5–10 cm
10–20 flowers per inflorescence	3–8 flowers per inflorescence	2–6 flowers per inflorescence
Calyx 5–9 mm, slightly to markedly inflated in flower	Calyx 5–7 mm, slightly inflated in flower	Calyx 5–7 mm, not inflated
Corolla tubular, 2.5–4 cm long, deep purple, largely glabrous but with hairs around the mouth of the tube	Corolla tubular to funnel-shaped, 2.6–4.0 cm, yellow with purple, interior glabrous, exterior with few to many hairs	Corolla funnel-shaped to campanulate, 2–3.5 cm, yellow-green, interior glabrous, exterior pubescent
Filaments 2.5–4 cm, hairy toward the base	Filaments 1.6–2.2 cm, with sparse hairs toward the base	Filaments 1.5–2 cm, glabrous or with sparse hairs at the base
Anthers 3–4 mm	Anthers 3.5–6 mm	Anthers 4–7 mm
Pollen 25–33 μ m (mean = 28) in diam.	Pollen 25–33 μ m (mean = 28) in diam.	Pollen 28–33 μ m (mean = 30) in diam.
Style 2–4 cm	Style 1.8–2.8 cm	Style 1.2–1.6 cm
Berry conical, 1.8–2.5 cm long, bottom third enveloped in fruiting calyx	Berry globose to slightly conical, 1–1.5 cm long ¹ , largely enveloped in fruiting calyx	Berry globose to slightly conical, 1–1.6 cm, largely enveloped in fruiting calyx

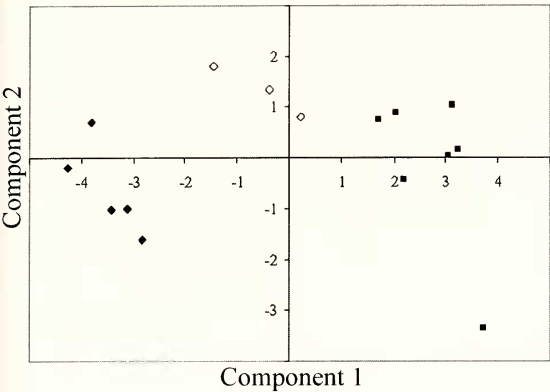


FIG. 3. Principal components analysis of morphological measurements from *I. cyaneum* (solid diamonds), *I. ayabacense* (open diamonds) and *I. lehmannii* (solid squares). Component 1 accounts for 60.7% of the variation, and component 2 for 12.7%.

I. lehmannii), suggesting that introgression remains possible. Also, *I. cyaneum*, *I. lehmannii* and *I. ayabacense* in Ayabaca are all pollinated by the same two species of hummingbird, *Adelomyia melanogenys* and *Coeligena iris* (Smith et al. 2008), suggesting that pollen flow could occur between the species when they occur in close proximity. However, individual hummingbirds appear to exhibit preference for particular *Ioichroma* species, thereby limiting interspecific pollen flow (Smith et al. 2008).

The frequency and evolutionary significance of interspecific hybridization in *Ioichroma* merits additional investigation. Within the last fifteen years, nine new species of *Ioichroma* have been described from the area of greatest overlap in species distributions, the Amotape-Huancabamba zone of Ecuador and Peru, and several new species have yet to be described (S. Leiva, HAO, pers. comm.). While a hybrid origin has been suggested for some of these taxa (Smith and Baum 2006), at least six of these new (described and undescribed) taxa have yet to be included in phylogenetic, morphological and biogeographic studies. Acknowledging the possibility of hybridization between *Ioichroma* species, it is imperative to consider the geographic distribution, the morphological distinctiveness and the stability of any newly-found form before granting it recognition as a new species.

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APPENDIX 1

HERBARIUM SPECIMENS USED FOR COMPARATIVE MORPHOLOGY

Vouchers for chromosome counts and pollen measurements are indicated with asterisks.

Iochroma ayabacense

*PERU, Piura, Ayabaca, carretera Ayabaca-Yacupampa, 4.6146°S 79.7118°W, *S. D. Smith, S. Leiva G. 337* (HAO, F, MO, NY, USM, WIS), 15/I/04.

PERU, Piura, Ayabaca, 4.6456°S 79.7192°W, *S. D. Smith, S. J. Hall 351* (HAO, F, MO, NY, USM, WIS), 16/I/04.

PERU, Piura, Ayabaca, Barrio San Jose Obrero, 4.6455°S 79.7178°W, *S. D. Smith 500* (QCNE, WIS), 30/I/05.

Iochroma cyaneum

*USA, Wisconsin, Madison, Grown from seed from a plant grown by W. G. D'Arcy at the Missouri Botanical Gardens, original collection likely to be Plowman 4594 from Azuay, Ecuador, *S. D. Smith 265* (WIS), 20/V/2003.

PERU, Piura, Ayabaca, carretera Ayabaca-Piura, 4.6514°S 79.7362°W, *S. D. Smith, S. Leiva G. 329* (HAO, F, MO, NY, USM, WIS), 14/I/04.

PERU, Piura, Ayabaca, Yacupampa, 4.6194°S 79.7106°W, *S. D. Smith, S. Leiva G. 336* (HAO, F, MO, NY, USM, WIS), 15/I/04.

PERU, Piura, Ayabaca, Barrio San Jose Obrero, 4.6441°S 79.7185°W, *S. D. Smith 502* (QCNE, WIS), 30/I/05.

ECUADOR, Loja, Cerro Sozoranga, 4.3553°S 79.7022°W, *P. M. Jorgensen, C. Ulloa, H. Vargas G. Abendano 628* (MO), 29/IV/1994.

Iochroma lehmannii

*PERU, Piura, Ayabaca, carretera Ayabaca-Piura, 4.6596°S 79.7404°W, *S. D. Smith, S. Leiva G. 330* (HAO, F, MO, NY, USM, WIS), 14/I/04.

PERU, Piura, Ayabaca, Bosque Cuyas, 4.6035°S 79.7110°W, *S. D. Smith, S. Leiva G. 339* (HAO, F, MO, NY, USM, WIS), 15/I/04.

PERU, Piura, Ayabaca, Bosque Cuyas, *S. Leiva G., N. W. Sawyer, V. Quipuscoa 2028* (F), 6/VI/97.

ECUADOR, Canar, Km 15 Chunchi-Zhud, 2.3480°S 78.9377°W, *S. D. Smith, L. Lopez 486* (F, QCNE, MO, WIS), 8/I/05.

ECUADOR, Canar, Km 20 Chunchi-Zhud, 2.3555°S 78.9590°W, *S. D. Smith, L. Lopez 487* (QCNE, MO, NY WIS), 8/I/05.

ECUADOR, Chimborazo, Carretera Alausi-BagUIL-Guamote, *V. Zak 2375* (MO), 11/VIII/1987.

ECUADOR, Canar, Km 21 Chunchi-Zhud, 2.4333°S 79.0333°W, *A. Alvarez and M. Tirado 1475* (MO), 9/VII/1995.

APPENDIX 2

CHARACTERS SCORED FOR PRINCIPAL COMPONENTS ANALYSIS

Leaf length
Leaf width
Pedicel length
Calyx width
Calyx length
Corolla width at base
Corolla width at mouth
Corolla length
Corolla pigmentation (0 = no purple, 1 = light purple, 2 = deep purple)
Corolla pubescence (0 = glabrous or few hairs, 1 = pubescent)
Style length
Filament length
Anther length
Flowers per inflorescence